

against the malaria antigen is currently being tested. The underlying assumption is that these MHC class I self-antigens constitute a part of the repertoire that some T cells 'see' as foreign. This has clearly been shown to be the case with Ly-2⁺ killer cells^{20,21} and there is evidence that increased expression and turnover of these class I antigens enhances both tumour immunity²² and killer cell activity against TNP-modified self²³. However, Ly-1⁺ helper cells have not been reported to use class I antigen to facilitate their interaction with foreign antigen. The Ly-1⁺ cells are the cells which confer protective immunity against malaria; no significant role for Ly-2⁺ cells has been found²⁴. There is some evidence that suppressor cells may see antigen together with class I antigens^{25,26}. Thus, it may be that the enhanced immunogenicity of parasitized reticulocytes is due to a negative effect on suppressor cells produced by the expression of high levels of class I antigens. This negative effect could be brought about either directly, by inhibiting their triggering or indirectly, by activating cells of the newly described contrasuppressor circuit²⁷.

That reticulocytes may provide a generally more immunogenic presentation of antigens derived from intracellular parasites is not itself a novel concept. Playfair *et al.*²⁸ used phenylhydrazine to induce reticulocytosis and found that when these cells were infected with either *P. yoelii* or *P. berghei* and subsequently labelled with TNP, they induced a better anti-TNP IgM response than did normal reticulocytes. These results indicate that parasitized reticulocytes present exogenous antigens in a more immunogenic fashion to the immune system and are consistent with our finding that they present the parasitic (malarial) antigens in a better fashion as well. This finding brings up the question of whether or not the parasitized reticulocytes bearing the fatal form of the *P. yoelii* also express the same increased amounts of H-2 antigens as do the reticulocytes parasitized with the nonfatal form. Playfair's data suggest that such might be the case since the parasitized reticulocytes presented TNP much better than did normal reticulocytes, indicating that the parasitic infection of the phenylhydrazine-induced reticulocytes made them better carriers of haptens. We are now trying to determine if it is simply the increased H-2 antigens on the reticulocytes that are responsible for their hyperactivity as carriers of antigen. However, even unparasitized reticulocytes carry more H-2 antigens than do mature red blood cells and could thus, in theory, present malaria antigens to the immune system in a more efficient manner.

These results strongly suggest that the type of cell in which malarial parasites grow is an important factor in determining the immune response against them. There is a very good correlation of the expression of high levels of class I MHC antigens with immunogenicity and, if a causal relationship can be found, these results could have far-reaching effects on the methods of preventive immunization, not only against malaria or other parasites, but perhaps also against other kinds of foreign material that would be advantageous for the body to reject.

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A survey of human leukaemias for sequences of a human retrovirus

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Human T-cell leukaemia-lymphoma virus (HTLV) is an exogenous human retrovirus distinct from all known animal retroviruses. HTLV is closely linked to a subtype of adult T-cell malignancies and except for isolated cases, has not been found associated with any other form of leukaemia, lymphoma or other cancers (see refs 1, 2 for review). HTLV can be transmitted to cord blood T lymphocytes *in vitro* and the infected cells exhibit characteristics of transformed neoplastic T cells³⁻⁵. We have recently cloned DNA sequences derived from approximately 1 kilobase (kb) of the 5' and 3' termini of the HTLV genome, as well as a 4-5-kb defective HTLV provirus flanked by cellular sequences⁶. The availability of these probes has enabled us to carry out a limited survey of different fresh or cultured cells from patients of different lymphoid and myeloid malignancies for HTLV-related DNA sequences. The results presented here show that cells from all Japanese patients with adult T-cell leukaemia and several patients with various mature T-cell malignancies from elsewhere contained one or more copies of a highly conserved HTLV genome. The infected cells are of clonal origin. Fresh cells from 1 of the 10 myeloid leukaemic patients contained exogenous DNA sequences distantly related to HTLV.

Four different viral probes which represent different regions of the HTLV genome were used: two recombinant plasmids containing 400 and 600 nucleotides, respectively, of R-U5 and R-U3 sequences, a 6-kb fragment of a cloned defective integrated HTLV provirus from the neoplastic T-cell line CR-M2 containing *LTR-env-pol* and 1 kb of flanking cellular sequences, and a 3-kb fragment containing only sequences of the *env-pol* region. Table 1 summarizes results of DNA from various mature and immature T- and B-cell lines digested with various restriction enzymes and blot-hybridized to different nick-translated probes. One of the enzymes used, *EcoRI*, does not cut within the prototype HTLV genome, thus each *EcoRI* band detected on a Southern blot by HTLV probes represents a single proviral copy at a particular integration site (ref. 7 and our unpublished data). All the mature T-cell lines known to express HTLV antigens and/or infectious virus contained one

or more HTLV genome copies. MO, a T-cell line developed from a patient with hairy cell leukaemia⁸, has been shown to produce a subtype of HTLV distinct from the prototype as demonstrated by the slopes of competitive immunoprecipitation of purified viral p24 (ref. 9). HTLV *env-pol* probes detect in MO DNA sequences which are distinguishable from prototype HTLV by restriction patterns and stringency of hybridization (not shown). Analysis of MO cell RNA in relaxed hybridization conditions revealed hybridizing viral messenger RNA (G.F. *et al.*, unpublished). These results suggest that HTLV from MO differs from the prototype HTLV at the nucleotide sequence level, although it belongs to the HTLV family because its target is also mature T cells and its proteins (p24, p19) are related to those of the prototype HTLV. Another cell line, HUT 78, of a mature T cell that expresses no HTLV antigens or virus particles contained HTLV proviral sequences (R.C.G. *et al.*, unpublished). Several other lymphoid cell lines, including several mature T-cell lines from patients with mycosis fungoides, Sézary syndrome, immature T-cell lines and B-cell lines failed to reveal any HTLV-related sequences (Table 1). Fresh leukaemic cells from patients with different lymphoid malignancies were screened as described above. DNA from all Japanese patients with adult T-cell leukaemia (ATL) examined (five out of five) contained one or two copies of HTLV provirus, including a patient (T.O.) who had no circulating antibodies against HTLV antigens (Robert-Guroff *et al.*, unpublished). On the other hand, a patient (E.T.) with T-cell acute lymphocytic leukaemia (T-ALL) was found to have antibodies reactive with HTLV antigens¹⁰ even though his leukaemic cells were immature T cells, normally not the target for HTLV infection. By hybridization, his leukaemic cells indeed lacked HTLV DNA sequences. This result suggests that this patient was at one time exposed to HTLV. His disease may have had a different aetiology, or viral sequences may have been deleted from his malignant cells in an event that also resulted in the loss of the mature T-cell markers. Other positive samples include leukaemic cells from an Israeli patient (U.K.) with peripheral diffuse lymphoma from whose cells a virus-producing line has been established^{4,10} (also see Table 1) and a skin biopsy of a Brazilian patient (M.A.) with mycosis fungoides. Several other samples from patients with Sézary syndrome and cutaneous T-cell lymphoma (CTCL), as well as 20 ALL and chronic lymphocytic leukaemia (CLL) samples were negative in this survey.

We conclude that all HTLV-positive fresh leukaemic cells and cultured cell lines are clonal populations of infected cells, because DNA bands greater than genomic size (which therefore must contain viral and flanking cellular sequences) are readily visible (Figs 1, 2). Figure 1 shows a representative DNA blot of samples from two ATL patients hybridized to a HTLV_{R-US}

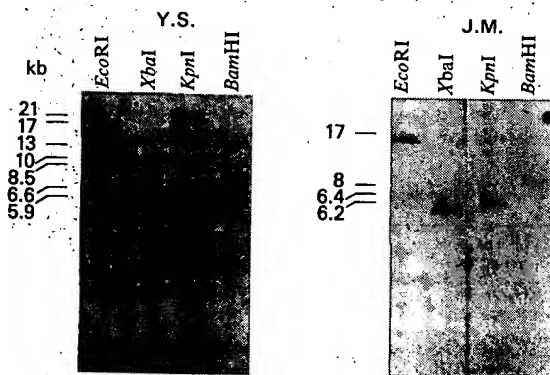


Fig. 1 Integration of HTLV proviruses in fresh leukaemic cells of two Japanese patients with adult T-cell leukaemia. 30 µg of cellular DNA from leukaemic cells of two ATL patients were digested with *EcoRI*, *XbaI*, *KpnI* and *BamHI* and analysed by electrophoresis in a 0.8% agarose gel. The gel was blotted onto nitrocellulose filter paper and the filter was hybridized to nick-translated ³²P-labelled HTLV_{R-US} sequences.

probe. DNA from Y.S. and J.M. contained two and one copies of HTLV, respectively, as shown by the number of *EcoRI* viral fragments. It is of interest that the enzymes *XbaI*, *KpnI* and *BamHI*, which cut within the prototype HTLV genome, generated a single DNA fragment in the J.M. sample. As the R-U5 probe detects sequences within the long terminal repeat (LTR), which are present twice in a complete provirus, our data suggest that J.M. contained a single defective provirus lacking at least one LTR. Defective proviruses or combinations of nondefective and defective proviruses have been found in avian leukosis virus-induced lymphomas^{11,12} and bovine leukaemia virus-associated lymphomas¹³. This result also indicates that not all leukaemic cells from HTLV-positive leukaemias will yield fully infectious viruses.

Apart from HTLV_{MO}, which is greatly divergent from the prototype HTLV⁹, all other HTLV isolates so far obtained seem to be closely related to each other in their proteins and nucleotide sequence homology. We had proposed to classify HTLV_{MO} as HTLV-II and all other HTLV strains as HTLV-I subgroups⁹. For a more sensitive comparison, we examined DNA from all the HTLV-positive fresh cell samples and cell lines derived from primary tumour cells by Southern hybridization, using the enzymes *BamHI* and *PstI*, which excise internal fragments from within HTLV_{CR} (ref. 6). As shown in Fig. 2, the HTLV genomes as present in patients from Japan, USA, Israel, Brazil and the Caribbean all contain an internal 1.05-kb *BamHI* fragment and a 2.6-kb *PstI* fragment as detected by the HTLV *env-pol* probe. This result indicates that the different HTLV strains of subgroup I are highly conserved throughout many geographical regions.

Although seroepidemiological studies revealed an association of HTLV strictly with T-cell malignancies and the prototype HTLV infects preferentially mature T cells *in vitro*, we were interested to see if cloned HTLV probes might detect cross-reactive sequences in cells of other haematopoietic neoplasias.

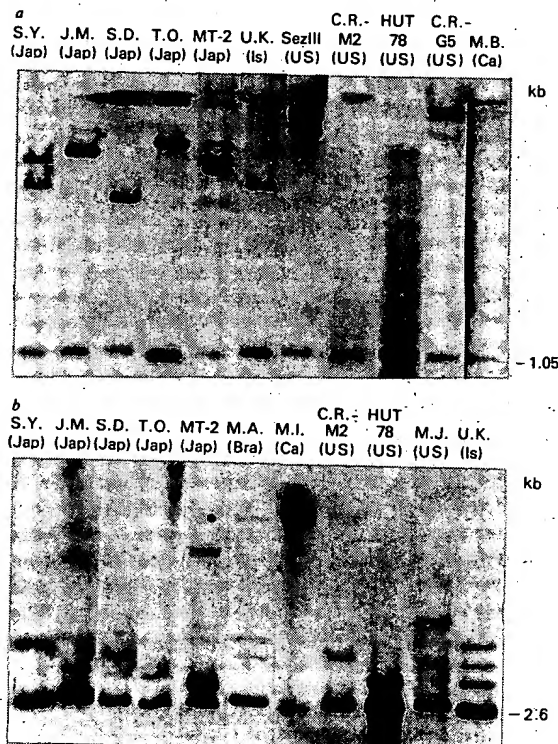


Fig. 2 Conservation of the HTLV genomes present in diverse geographical areas. DNA from fresh leukaemic cells or cell lines from HTLV-positive patients from different areas of the world was digested with the restriction enzymes *BamHI* (a) and *PstI* (b) which cut more than once within HTLV_{CR}, and blot hybridized to a HTLV *env-pol* probe. The places of origin are: Jap, Japan; Is, Israel; US, United States; Ca, Caribbean; Bra, Brazil.

We examined fresh leukaemic cells from 10 patients with acute (AML) and chronic (CML) myelogenous leukaemia. All except one sample were negative. DNA from fresh cells of one CML patient in blast crisis revealed a 4.7-kb *Xba*I band and a 10-kb *Kpn*I band hybridized to HTLV LTR probes after a 2-week exposure of the autoradiogram (Fig. 3). A duplicate filter hybridized to pHT-3; a recombinant plasmid containing a single copy cellular gene¹⁴, yielded a different array of bands characteristic for pHT-3, indicating the bands detected by HTLV-LTR were not due to pBR322 cross-hybridizing sequences (Fig. 3). However, HTLV probes that lack LTR sequences did not hybridize to this DNA (not shown). This result suggests several possibilities: (1) the leukaemic cells from this patient contained

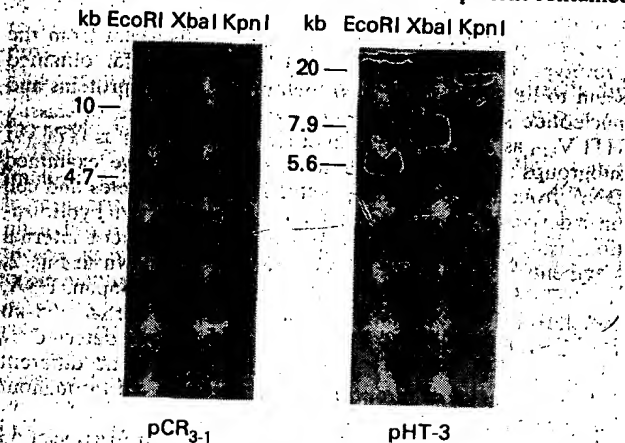


Fig. 3 Detection of HTLV-related DNA sequences in leukaemic cells of a CML patient. Duplicate DNA filters were hybridized with labelled PCR-31, a recombinant plasmid containing HTLV_{R-US} sequences (a) and pHT-3, a recombinant plasmid containing a single copy cellular gene¹⁴ (b).

Table 1 Survey of human lymphoid cell lines for HTLV provirus

Cell line	Diagnosis	Cell type	HTLV markers	Proviral copy no.
HTLV positive				
CR-M2	CTCL (MF)	Mature T cells	+	2-3
CR-G5	CTCL (MF)	Mature T cells	+	1
HUT-78	CTCL	Mature T cells	-	1
SEZ III	CTCL	Mature T cells	+	3
MB	CTCL	Mature T cells	+	1
MI	T-LCL	Mature T cells	+	>3
UK	PTCL	Mature T cells	+	2
MJ	CTCL (MF)	Mature T cells	+	>3
WA	DML	Mature T cells	+	1
MT-2	ATL	Mature T cells	+	>3
TK	PL	Mature T cells	+	>3
BK	Healthy	Mature T cells	+	1
HK	Healthy	Mature T cells	+	>3
MO	THCL	Mature T cells	+	>1
HTLV negative				
HPB-MLT	T-LML	Immature T cells	-	-
HPB-ALL	T-ALL	Immature T cells	-	-
CR-B	CTCL (MF)	B cells	-	-
8392	T-ALL	B cells	-	-
8402	T-ALL	Immature T cells	-	-
CCR-SB	T-ALL	B cells	-	-
HSB2	T-ALL	Immature T cells	-	-

CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; T-LCL, T-lymphosarcoma cell leukaemia; PTCL, peripheral T-cell lymphoma; DML, diffuse mixed lymphoma; ATL, adult T-cell leukaemia; PL, persistent lymphocytosis; T-LML, leukaemic phase of T-cell lymphoma; T-ALL, T-cell acute lymphocytic leukaemia; THCL, T-cell variant of hairy cell leukaemia. T.K., B.K. and H.K. are family members (mother, father and brother, respectively) of a Japanese ATL patient, S.K.¹⁶ MO is releasing a virus that is distantly related to the prototype HTLV (see text). As this virus has related nucleic acid sequences, but a different restriction enzyme map, the restriction mapping criteria for integrated proviral copies cannot be applied.

a small population of T cells which harboured HTLV; (2) HTLV is not entirely T-cell tropic and is involved in some myeloid malignancies; (3) the HTLV probe is detecting cross-reactive sequences of a distinct retrovirus associated with some myeloid leukaemias. The weak signal intensity obtained only with HTLV-LTR sequences and the apparent monoclonality of the infected cells tend to support the last possibility.

Thus, we have used cloned HTLV nucleic acid sequences to survey a number of human haematopoietic cell lines and fresh leukaemic cells from patients with different lymphoid and myeloid malignancies for DNA sequences related to HTLV. We draw several conclusions from these studies:

(1) Cells from some patients of mature T-cell malignancies, including all patients with ATL, contained one or a few copies of HTLV provirus. In some cases, a single defective provirus was present, suggesting that not all HTLV-positive leukaemic cells would yield fully infectious virus.

(2) The surveys by molecular hybridization and by serology do not correlate in every case. We believe this discrepancy to be due to the following situations: patients whose leukaemic cells have proviral sequences but do not express viral antigens (for example, due to defective viral genomes) and thus do not produce antiviral antibodies; alternatively, patients who have incidental HTLV infection of their T lymphocytes or some other target cell and are therefore positive for antibodies, but whose leukaemic cells do not contain integrated HTLV proviral sequences and therefore whose disease is probably not linked to HTLV.

(3) The HTLV strains can be divided into two subgroups. HTLV-I is a virus whose sequences are highly conserved despite extensive distribution throughout different regions of the world. To date, HTLV-II has only one isolate, the MO strain, which differs from HTLV-I in sequence homology and restriction enzyme mapping.

(4) The tumour cells are clonal expansions of single infected cells. Monoclonality is a common feature of tumours induced by the chronic leukaemia viruses, but not by the rapidly transforming *onc* gene-containing viruses. It has been proposed that activation of cellular genes is one mechanism of leukaemogenesis by the chronic leukaemia viruses¹⁵. Activation of a cellular gene (HT-3) specifically involved in mature T-cell proliferation was observed in all HTLV-infected cells¹⁴. It would be of great interest to determine whether there is a common integration locus for HTLV and whether activation of cellular genes, including HT-3, occurs in the vicinity of the provirus integration sites.

(5) We detected weakly cross-reactive sequences, which may represent a distantly related virus, in cells from one CML patient. This result suggests that although the prototype HTLV is specifically associated with mature T-cell malignancies, cloned HTLV sequences may be useful in detection of other cross-reactive putative human retroviruses involved in other malignancies.

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